Available online on 15.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Formulation Development and Evaluation of Herbal Toothpaste for Treatment of Oral Disease

Kavita Varma Shukla, Deepika Kumari*

Shri Ram Institute of Pharmacy, Madhotal, Jabalpur, Madhya Pradesh, 482002, India

ABSTRACT

Streptococcus mutans is the most common cause of tooth decay. Parabens and other commonly used as anti-*Streptococcus* agents in toothpaste industry have numerous side effects such as discoloration of teeth. The herbal extract of all three plants gives antimicrobial and anti-inflammatory activity and prevent and reducing the tooth decay, dental caries and given to freshness of mouth. The aim of present work was development and evaluation of herbal antimicrobial toothpaste containing Bark of *Acacia nilotica, Acacia catechu* and flower buds of *syzygium aromaticum* as herbal ingredients. Different types of formulations (F1-F6) were formulated using calcium carbonate as abrasive and Glycerine as humectant in varied concentrations. All the formulations were evaluated for various parameters like dryness, color, appearance, consistency, washability, pH, spreadability and foaming power. Polyherbal toothpaste containing hydroalcoholic extract of plants was tested for antimicrobial activity against *Staphylococcus aureus* and *Streptococcus mutans* with different concentrations of toothpaste were used (25, 50and 100 mg/ml). Among the tested bacteria used *Staphylococcus aureus* was found to be most sensitive to the formulated toothpaste as seen by zone of inhibition (19-24 mm) followed by *Streptococcus mutans* (13-18 mm). The results showed that the formulated polyherbal toothpaste is promising antimicrobial effects against both organisms. It may be safer compared to fully synthetic toothpaste. Further studies are warranted to prove safety and efficacy of the formulated polyherbal toothpaste.

Keywords: *Streptococcus mutans, Acacia nilotica, Acacia catechu, syzygium aromaticum,* polyherbal toothpaste

Article Info: Received 09 June 2019; Review Completed 19 July 2019; Accepted 20 July 2019; Available online 15 August 2019

Cite this article as:

Shukla KV, Kumari D, Formulation Development and Evaluation of Herbal Toothpaste for Treatment of Oral Disease, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):98-104 **http://dx.doi.org/10.22270/jddt.v9i4-s.3344**

***Address for Correspondence:**

Deepika Kumari, Shri Ram Institute of Pharmacy, Madhotal, Jabalpur, Madhya Pradesh, 482002, India

INTRODUCTION

Herbal medicines are referred to the use any part of the plants for healing and treating diseases purposes. Herbal medicines have been used widely throughout human history and according to World Health Organization (WHO) about 80% of the human population used herbal medicine for primary healthcare[1]. In addition, more than 35,000 plant species have been reported to be used in various human cultures around the world for medical purposes[2]. Some of them are potent antimicrobial, antidiabetic, antiviral, anticancer and antifungal. The oral cavity infections are the most common types of infections. Dental caries, an infectious disease, results in damage and infection of enamel and dentine[3]. If left untreated, the infection continues and will lead to tooth loss. The mouth normal flora consists of opportunistic bacteria which are normally non-pathogenic. The imbalance of this situation creates infection and tooth decay. *Streptococcus mutans* is considered as the main species involved in the development of dental caries[4]. *Streptococcus mutans,* acid producing bacteria, causes fermentation of carbohydrates which results in tooth decay[5]. Toothpaste, toothbrush and mouthwash that contain antimicrobial agents are commonly used as products which improve oral hygiene. Their uses dates back to ancient times and continues up to now[6].Toothpaste, as an irreplaceable agent in effective home care system, is a gel or paste dentifrice used with a toothbrush as an accessory to clean and maintain health of teeth in order to enhance oral hygiene[7]. *Acacia* is the most significant genus of family: Mimosaceae first of all described by Linnaeus in 1773. It is estimated that there are roughly 1380 species of *Acacia* worldwide, about two third of them native to Australia and rest of spread around tropical and subtropical regions of the world[8]. *Acacia nilotica* was used by herbalist to treat colds, diarrhea, dysentery, inflammation, itch, measles, sore throat, wounds and Sexual Transmitted Disease. The plant also possessed antiemetic, antibacterial, expectorant and antiseptic activities. Its juice is used in folk remedies for the cancerous condition. Pain and inflammation are common complaints in many patients suffering from acute conditions[9,10]. Acacia catechu, a deciduous tree of the Fabaceae family is indigenous in India, other Asian countries, and East Africa. It is commonly known as catechu, cachou

and black cutch. This plant is widely used in Ayurveda for many diseases including skin diseases. Ayurveda uses bark and heartwood of this plant for various formulations. Khadirarishta is a famous Ayurvedic skin tonic prepared from A. catechu. Khadira Sara, the heartwood extract is used as ingredient in many medicines such as Lavangadi Vati[11]. *Syzygium aromaticum* (clove) is one of the most valuable spices that have been used from centuries as food preservative and for many medicinal purposes. Nowadays cloves are cultivated in several parts of the World[12]. *Syzygium* species (Fam. Myrtaceae) have been reported to possess biological activities[13]. Clove's Botanical name is *Caryophyllus aromaticus* which is derived from the Latin "clavus", which means nail due to its resemblance with the shape. The clove tree is an evergreen tropical plant, which flowers twice every year. Cloves are the unopened buds and harvested when the outer green leaves have changed from green to a yellow pink[14]. The cloves are highly antiseptic[15], antimutagenic, anti-inflammatory, antioxidant, anti-ulcerogenic, antithrombotic, anti-parasitic[16], antibacterial[17], antifungal[18] and antiviral[19]. Bud oil of clove has natural behavior and the main properties include antioxidant, insecticidal, antifungal and antibacterial properties[20].

MATERIALS AND METHODS

Plant material

Bark of *Acacia nilotica, Acacia catechu* and flower buds of *syzygium aromaticum* was collected from local area of Bhopal in the month of May, 2019.

Extraction by maceration process

64 gm of *Acacia nilotica* and 52 gm of *Acacia catechu* and *syzygium aromaticum* dried powdered has been extracted with hydroalcoholic solvent (Ethanol: Water: 80:20) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40ºC.The extract was evaporated above their boiling points. The extracts were stored in air-tight containers in the refrigerator at 4 ^{o}C until further use.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Qualitative phytochemical analysis of plant extract

The extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate[21,22]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso et al[23]. A volume of 2 ml of extracts or standard was mixed with 5 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso et al[23]. 1 ml of 2% AlCl3 methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

Total alkaloids content estimation

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

Method of preparation of herbal tooth paste

Firstly gum is mixed with humectants for proper dispersion. Other powdered ingredients are shifted together and added gradually to mucilaginous mixture with continuously gentle stirring. The aqueous media and extracts are mixed and stirred to get product detergent flavors and other ingredients are added.

Table 1 Development of the formulation (toothpaste)

Formulation Code (g/100gm)	F1	F2	F3	F4	F5	F6
Hydroalcoholic extract of (AC)	0.25	0.25	0.25	0.25	0.25	0.25
Hydroalcoholic extract of (AN)	1	1	1	1	1	1
Hydroalcoholic extract of (SA)	1	1	1	1	1	1
Calcium carbonate (gm)	21	23	25	21	23	25
Dicalcium Phosphate	24	25	26	24	25	26
Magnesium hydroxide (gm)	1	1	1	1	1	1
Sodim lauryl sulphate (gm)	0.5	1	1.5	0.5	1	1.5
Glycerine (gm)	30	30	30	30	30	30
Steivia (gm)	0.5	0.5	0.5	0.5	0.5	0.5
Methyl Paraben (gm)	0.1	0.1	0.1	0.1	0.1	0.1
Propyl Paraben (gm)	0.3	0.3	0.3	0.3	0.3	0.3
Water (gm)	100	100	100	100	100	100

AC- *Acacia catechu*, AN-*Acacia nilotica, SA- Syzygium aromaticum*

Packing

It is packed in a collapsible tube and at last sealed with the help of collapsible tube sealing machine.

Evaluation of herbal toothpaste

Organoleptic evaluation

Organoleptic evaluation (colour, taste) was done by sensory and visual inspection.

pH

pH was tested by dissolving 1 gm product in to 9 ml of water and shaked vigorously then aqueous solution and pH is observed by pH meter.

Fragrance test

It was based on individual observation for its acceptability.5 people were asked for acceptability of fragrance and their opinion was taken. And fragrance was evaluated based on the below-described criteria;

- A). the fragrance was good, as good as the fragrance of reference toothpaste.
- B). the fragrance was not so good but comparable to the reference toothpaste.
- C). the fragrance of the toothpaste was poor than the reference toothpaste.

Shape retention

Tooth paste was squeezed out from the tube and put entirely of a tooth brush and the state of the toothpaste after it was allowed to stand for 10 seconds was evaluated based on the below-described criteria;

- A).Shape just after the toothpaste is squeezed out on the toothbrush is maintained.
- B). Shape just after the toothpaste is squeezed out on the toothbrush is almost maintained.
- C). the toothpaste squeezed from the toothbrush and cannot maintain its shape.

Moisture content

Toothpaste (10 gm) weighted in a Porcelain dish and dried it in the oven at 105°C. It was cooled in a desiccater. The loss of weight is recorded as percentage moisture content and calculated by the given formula.

% Moisture = Original sample weight – dry sample weight/ Original sample weight

Foaming character

1) 1 gm of tooth paste was poured into stoppered test tube (height 16 cm. diameter 6 mm) and volume of the liquid was adjusted with the water up to 10 ml. Tube was stoppered and shaked length wish, motion for 16 second, two shake/second. Allowed to stand for 15 minutes and height of the foam produced was measured.

2) .10% solution of tooth paste was prepared. 4ml of this solution was added to146 ml of water at 30°C .The solution was agitated for 10 seconds. The foam was poured in to a 100 ml graduated cylinder to overflowing. A rubber stopper was gently dropped in to the foam. The time for the rubber stopper to pass two points (40ml-80ml) was measured. Longer time of fall indicates the denser and more stable foam.

Threading property

The threading property of each toothpaste when it was squeezed out on the entirely of a toothbrush and slowly pulled up was evaluated based on the below-described criteria.

Evaluation criteria of threading property;

- A).The toothpaste can be put on a toothbrush smoothly without threading.
- B).The toothpaste can be put on a toothbrush smoothly, though it causes slight threading.
- C) The toothpaste cannot be put on toothpaste smoothly because of severe threading.

Storage stability

The toothpaste were filled in a toothpaste tube for storage and stored for 45 days at each of 5°C, room temperature and 40°C. The tube was then cut through and whether the liquid component was separated from the toothpaste or not was evaluated based on following criteria.

Evaluation criteria of storage stability;

- A). Separation of a liquid component is not observed at all.
- B). Separation of a liquid component is observed slightly.
- C). Separation of a liquid component is observed obviously.

Net content: net content was calculated by using following formula;

Net content = weight of filled tube – weight of empty tube.

Total flavonoid content estimation of Tooth paste Formulation

1 ml of 2% $AlCl_3$ solution was added to 3 ml of stock solution of tooth paste and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Stability study (Storage stability)

Toothpaste was stored at 40°C and RH 75% ± 5% for 45 days. Estimation of Flavonoids was performed at zero period and then samples were withdrawn after every 9 days, total 5 samples were withdrawn. Toothpaste (1 gm) was refluxed with distilled water (75 ml) for 30 min. for complete extraction of flavonoids and filtered through sintered glass funnel by vacuum filtration assembly. The filtrate was centrifuged at 2000 rpm for 20 minutes, the supernatant was collected in 100 ml volumetric flask and volume was made up with water.

The same procedure was performed for each sample and solutions (100 ml) of their Total flavonoids content were determined.

Antimicrobial activity of toothpaste

The well diffusion method was used to determine the antimicrobial activity of the Toothpaste using standard procedure of Bauer *et al*[24].The drug used in standard preparation was ofloxacin and ciprofloxacin of IP grade. The antimicrobial activity was performed by using 24hr culture of *S. Mutans and S. aureus.* There were 3 concentration used which are 25, 50 and 100mg/ml for each extracted phytochemicals in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as

an inoculums. The plates were incubated at 37°C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug. The diameter of zone of inhibition of each wall was recorded.

RESULTS AND DISCUSSION

The crude extracts so obtained after maceration extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the plants using Pet ether and hydro alcohol as solvents are depicted in the Table 2. The results of qualitative phytochemical analysis of the extract were shown in Table 3-5. Hydroalcoholic extracts of plants showed the presence of saponins, diterpenes, phenols, and flavonoids. Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) total flavonoid content (TFC) and total alkaloid content (TAC) Table 6. The formulated toothpaste was evaluated for physicochemical parameters such as consistency, colour, odour, taste and pH. The results were shown in table7. Toothpaste formulated as semi-solid in consistency polyherbal toothpaste was brown in colour and fragrance was mood elevating and very pleasant in taste. As shown in the table 8, the herbal toothpaste has so many features, it exhibit good shape retention and threading preventive effect and a pleasant and mood elevating fragrance as compared to the reference toothpaste. 1 gm of tooth paste was poured into stoppered test tube (height 16 cm. diameter 6 mm) and volume of the liquid was adjusted with the water up to 10 ml. Tube was stoppered and shaked length wish, motion for 16 second, two shake/second. Allowed to stand for 15 minutes and height of the foam produced was measured this experiment was repeated three times and the results are assessed. Height of the foam was produced more than 1 cm every time. This indicates stable foam. 10% solution of tooth paste was prepared. 4ml of this solution was added to146 ml of water at 30°C .The solution was agitated for 10 seconds. The foam was poured in to a 100 ml graduated cylinder to overflowing. A rubber stopper was gently dropped in to the foam. The time for the rubber stopper to pass two points (40ml-80ml) was measured Table 9. Net content of toothpaste was 20 gm. Total flavonoids and moisture content estimation of formulated toothpaste of all formulation was given in Table 10. On the basis of all parameter check formulation F3 found to be good so storage stability study of optimized formulation F3 was given in Table 11. The formulated toothpaste was tested for antimicrobial activity against *Staphylococcus aureus,* and *Streptococcus mutans* with different concentrations of toothpaste were used (25, 50 and 100 mg/ml). The potency was qualitatively and quantitatively assessed by the presence or absence of a zone of inhibition and zone diameter values. Different concentrations show different reading in terms of zone of inhibition. The formulated toothpaste exhibited highly significant effect towards all the tested bacteria, Among the tested bacteria used *Staphylococcus aureus* was found to be most sensitive to the formulated toothpaste as seen by a zone of inhibition (19-24 mm) followed by *Streptococcus mutans* (13-18 mm) result are given in Table 12,13.

Table 2 % Yield of plant material

S. No.	Solvents	*Acacia nilotica*	*Acacia catechu*	*Syzygium aromaticum*
1	Pet ether	4.31	3.97	3.65
2.	Hydroalcoholic	8.55	7.21	7.88

Table 3 Phytochemical screening of extract *of Acacia nilotica*

S. No.	Phytochemicals	Tests	Observation	Inference
1.	Alkaloids	Mayer's Test	No Blue coloured	-
		Wagner's Test	No reddish brown precipitated	-
		Dragendorff's Tests	No orange brown precipitated	-
		Hager's Test	No Yellow coloured precipitated	-
2.	Flavonoids	Alkaline Reagent Test	Colourless	+
		Lead acetate Test	Yellow coloured precipitate	+
3.	Glycosides	Modified Borntrager's Test	No Rose-pink coloured	-
		Legal's Test	No Pink to blood red coloured	-
4.	Phenols	Ferric Chloride Test	bluish black colour ed	+
5.	Saponins	Foam Test	Layer of foam	+
		Froth Test	Layer of foam	+
6.	Tannins	Gelatin Test	No white precipitated	-
7.	Carbohydrates	Molisch's test	No violet coloured	-
		Fehling's test	Reddish orange precipitated	+
		Benedict's Test	No Orange red precipitated	-
8.	Proteins and aminoacids	Xanthoproteic Test	yellow coloured	+
		Ninhydrin Test	No blue coloured	-
9.	Diterpenes	Copper acetate Test	emerald green coloured	+

Table 4 Phytochemical screening of extract *of Acacia catechu*

S. No.	Phytochemicals	Tests	Observation	Inference
1.	Alkaloids	Mayer's Test	No Blue coloured	-
		Wagner's Test	No reddish brown precipitated	-
		Dragendorff's Tests	No orange brown precipitated	-
		Hager's Test	No Yellow coloured precipitated	-
2.	Flavonoids	Alkaline Reagent Test	Colourless	+
		Lead acetate Test	Yellow coloured precipitate	+
3.	Glycosides	Modified Borntrager's Test	No Rose-pink coloured	-
		Legal's Test	No Pink to blood red coloured	-
4.	Phenols	Ferric Chloride Test	bluish black colour ed	+
5.	Saponins	Foam Test	Layer of foam	+
		Froth Test	Layer of foam	+
6.	Tannins	Gelatin Test	white precipitated	+
7.	Carbohydrates	Molisch's test	No violet coloured	-
		Fehling's test	Reddish orange precipitated	+
		Benedict's Test	No Orange red precipitated	-
8.	Proteins and aminoacids	Xanthoproteic Test	No yellow coloured	-
		Ninhydrin Test	No blue coloured	-
9.	Diterpenes	Copper acetate Test	emerald green coloured	-

Table 5 Phytochemical screening *of Syzygium aromaticum*

S. No.	Phytochemicals	Tests	Observation	Inference
1.	Alkaloids	Mayer's Test	No Blue coloured	-
		Wagner's Test	Reddish brown precipitated	+
		Dragendorff's Tests	No orange brown precipitated	-
		Hager's Test	Yellow coloured precipitated	+
2.	Flavonoids	Alkaline Reagent Test	Colourless	+
		Lead acetate Test	Yellow coloured precipitate	+
3.	Glycosides	Modified Borntrager's Test	No Rose-pink coloured	-
		Legal's Test	Pink to blood red coloured	+
4.	Phenols	Ferric Chloride Test	bluish black colour ed	+
5.	Saponins	Foam Test	Layer of foam	+
		Froth Test	Layer of foam	+
6.	Tannins	Gelatin Test	No white precipitated	-
7.	Carbohydrates	Molisch's test	No violet coloured	-
		Fehling's test	No Reddish orange precipitated	-
		Benedict's Test	No Orange red precipitated	-
8.	Proteins and aminoacids	Xanthoproteic Test	No yellow coloured	-
		Ninhydrin Test	No blue coloured	-
9.	Diterpenes	Copper acetate Test	No emerald green coloured	-

Table 6 Estimation of total phenolic, flavonoids and alkaloid content

S. No.	Hydroalcoholic extract	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	*Acacia nilotica*	0.879	0.987	-
2.	*Acacia catechu*	0.761	0.876	-
3.	*Syzygium aromaticum*	0.543	1.023	0.132

Table 7 Evaluation of physical characteristics of herbal toothpaste

Toothpastes Formulation	Consistency	Colour	Odour	Taste	pH*
F1	Semi-solid	Brown	Mood elevating	pleasant	6.8
F2	Semi- solid	Brown	Mood elevating	Pleasant	6.9
F3	Semi-solid	Brown	Mood elevating	pleasant	7.0
F4	Semi- solid	Brown	Mood elevating	Pleasant	6.9
F5	Semi-solid	Brown	Mood elevating	pleasant	6.8
F6	Semi- solid	Brown	Mood elevating	Pleasant	6.9

*Average of three determination (N=3)

Table 8 Evaluation of toothpaste on different parameters

S. no.	Evaluation parameter	Grades on the basis of evaluation criteria						Reference grade
1	Fragrance	F1	F2	F3	F4	F5	F6	
		A	A	A	A	A	A	A
2	Threading property	A	A	B	B	A	A	A
3	Shape retention	A	A	A	A	A	A	A

Table 9: Foam stability of tooth paste

S. No.	Time taken by rubber stopper to pass two points (40-80 ml) in min.					
	F1	F2	F3	F4	F5	F6
1	2.8	3.2	4.3	2.4	2.5	2.3
2	2.9	3.3	4.2	2.6	2.6	2.4
3	2.9	3.5	4.1	2.5	2.4	2.5

Table 10 Total flavonoids and moisture content estimation

S. No.	In House Tooth paste formulation	Concentration Found (mg/1000mg)	Percentage Moisture content
1.	F1	0.125	12.25
2.	F2	0.136	14.56
3.	F3	0.189	15.65
4.	F4	0.105	14.98
5.	F5	0.098	13.36
6.	F6	0.102	15.45

Table 11 Evaluation of storage stability of F3

S. no.	Storage stability 45 days	Grades on the basis of evaluation criteria									Reference grade
		F1	F2	F3	F4	F5	F6	F7	F8	F9	
1	at 40°C	B	B	B	B	B	B	B	B	B	B
2	at room tem.	A	A	A	A	A	A	A	A	A	A
3	at 5°C	A	A	A	A	A	A	A	A	A	A

Table 12 Antimicrobial activity of *standard drug* against selected microbes

S.N	Name of drug	Microbes	Zone of inhibition		
			10 µg/ml	20 µg/ml	30 µg/ml
1	Ciprofloxacin	*Staphylococcus aureus*	17±1.69	18±2.62	22±2.16
2	Ofloxacin	*Streptococcus mutans*	12±0.15	15±0.13	17±0.19

Table 13 Antimicrobial activity of herbal tooth paste against selected microbes

S. No.	Name of microbes	Zone of inhibition		
		Herbal tooth paste		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Staphylococcus aureus*	19±0.471	20±0.942	24±0.816
2.	*Streptococcus mutans*	13±0	15±1.88	18±2.62

CONCLUSION

Herbal toothpastes have an emphasized role in maintaining the oral hygienic nature as well as preventing dental caries. The formulated polyherbal toothpaste was successfully evaluated using different standard parameters including antimicrobial properties. The extract showed promising antimicrobial effects against both organisms. The formulated toothpaste may be safer compared to fully synthetic toothpaste. Further studies are warranted to prove safety and efficacy of the formulated toothpaste.

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